



LISTS OF SPECIES

Check List 12(4): 1925, 13 July 2016 doi: http://dx.doi.org/10.15560/12.4.1925 ISSN 1809-127X © 2016 Check List and Authors

Emballonuridae Gervais, 1855 (Chiroptera) of Reserva Biológica de Saltinho (Atlantic Forest), in Brazil, revealed by echolocation

Frederico Hintze¹, Eder Barbier^{1, 2} and Enrico Bernard^{1*}

- 1 Laboratório de Ciência Aplicada à Conservação da Biodiversidade, Departamento de Zoologia, Universidade Federal de Pernambuco, Rua Professor Nelson Chaves s/n, Cidade Universitaria, CEP 50670-420, Recife, PE, Brazil
- 2 Programa de Pós-graduação em Biologia Animal, Departamento de Zoologia, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, 1235, CEP 50670-901, Recife, PE, Brazil

1

* Corresponding author. E-mail: enrico.bernard@ufpe.br

Abstract: Seventeen species of emballonurid bats are known in Brazil, but their distribution is often determined by patchy records. However, due to almost species-specific calls, echolocation can help to refine the distribution of emballonurids. Here we use acoustic samplings to assess and complement the list of emballonurids of Reserva Biológica Saltinho, an important Atlantic Forest remnant in Pernambuco state, Northeastern Brazil. We positively matched calls of Saccopteryx bilineata (Temminck, 1838) that was foraging along forest edges. However, a series of calls significantly different from those emitted by S. bilineata indicate the presence of a second *Saccopteryx* sonotype in the area. The lower frequencies in this sonotype presumably came from a larger species, indicating the possible existence of an undescribed cryptic species of Saccopteryx. We also detected Centronycteris maximiliani (Fischer, 1829), recorded 35 years after its first record in Pernambuco, and an undetermined species of *Peropteryx* Peters, 1867. Our data proved that echolocation is a very useful technique for inventorying poorly known and hard-to-capture emballonurid species, with the potential to reveal the cryptic richness.

Key words: bioacoustics; *Centronycteris maximiliani*; cryptic species; emballonurids; Pernambuco; *Peropteryx*; *Saccopteryx bilineata*

INTRODUCTION

Found in tropical and subtropical regions, 54 species of bats belonging to the family Emballonuridae are currently known (Simmons et al. 2005; Goodman et al. 2006, 2008; Lim et al. 2010). Among those, 17 are recognized in Brazil (Nogueira et al. 2014). Like other insectivorous bats, the precise distribution of emballonurids is frequently underestimated because

they often avoid mist-nets or fly far from their reach on ground level (Rydell et al. 2002; Kunz and Parsons 2009). However, due to almost species-specific calls, the use of echolocation recording techniques make detection and identification of emballonurids possible and less costly than mist-netting (Rydell et al. 2002; Jung et al. 2007). Of all Neotropical bats, echolocation is probably more studied in emballonurids (e.g., Barclay 1983; Kalko 1995; Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013). Calls from this family are narrowband and multi-harmonic with an evident *quasi-*constant frequency (qCF) portion, and the frequency of maximum energy (FME) is present on the second harmonic (Jones and Teeling 2006; Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013).

As the recording of calls became more accessible (Adams et al. 2012), echolocation techniques are now being used for multiple purposes, from simply detecting the presence of bats (Estrada et al. 2004; Ford et al. 2006) to more elaborated approaches for studying bat foraging behavior (Britton and Jones 1999; Greif and Siemers 2010), niche differentiation (Sattler et al. 2007; Jacobs and Barclay 2009) or species identification (Russo and Jones 2002; Barataud et al. 2013; Horta et al. 2015). Specifically for species identification, several cryptic species of bats have been discovered based on their echolocation calls alone, or in combination with morphology and genetics (Jones and Parijs 1993; von Helversen et al. 2001; Jacobs et al. 2006; Ramasindrazana et al. 2011). For example, in Europe, Jones and Parijs (1993) linked one of the two different phonic types known for Pipistrellus pipistrellus (Schreber, 1774) to a potentially new cryptic species. The 45 kHz phonic type (sonotype) corresponds to P. pipistrellus, while the 55 kHz phonic type to P. pygmaeus (Leach, 1825) (Jones and Barratt 1999; Sztencel-Jablonka et al. 2009). In the western part of the Guianan Shield, an analysis of 200 acoustically identified individuals of *Pteronotus* parnellii (Gray, 1843) showed that two phonic types live in sympatry with no overlap in frequencies of their echolocation calls, with further molecular comparisons suggesting that the higher phonic type (~59 kHz) represented an undescribed species (Thoisy et al. 2014). Therefore, the recording and analysis of echolocation calls are a useful technique to study bat species identity and richness, especially for poorly known areas.

Here we use acoustic samplings to identify the emballonurids of Reserva Biológica Saltinho, an important Atlantic Forest remnant in Pernambuco state, Northeastern Brazil. We describe those sonotypes and explore the possible existence of an undescribed cryptic species for Brazil.

MATERIALS AND METHODS Study site

This study was performed in the Reserva Biológica de Saltinho (hereafter Saltinho; 08°43′49.32″ S, 035° 10′34.95″ W, datum WGS84) (Figure 1), a 562 ha federal reserve of Atlantic Forest, near Tamandaré,

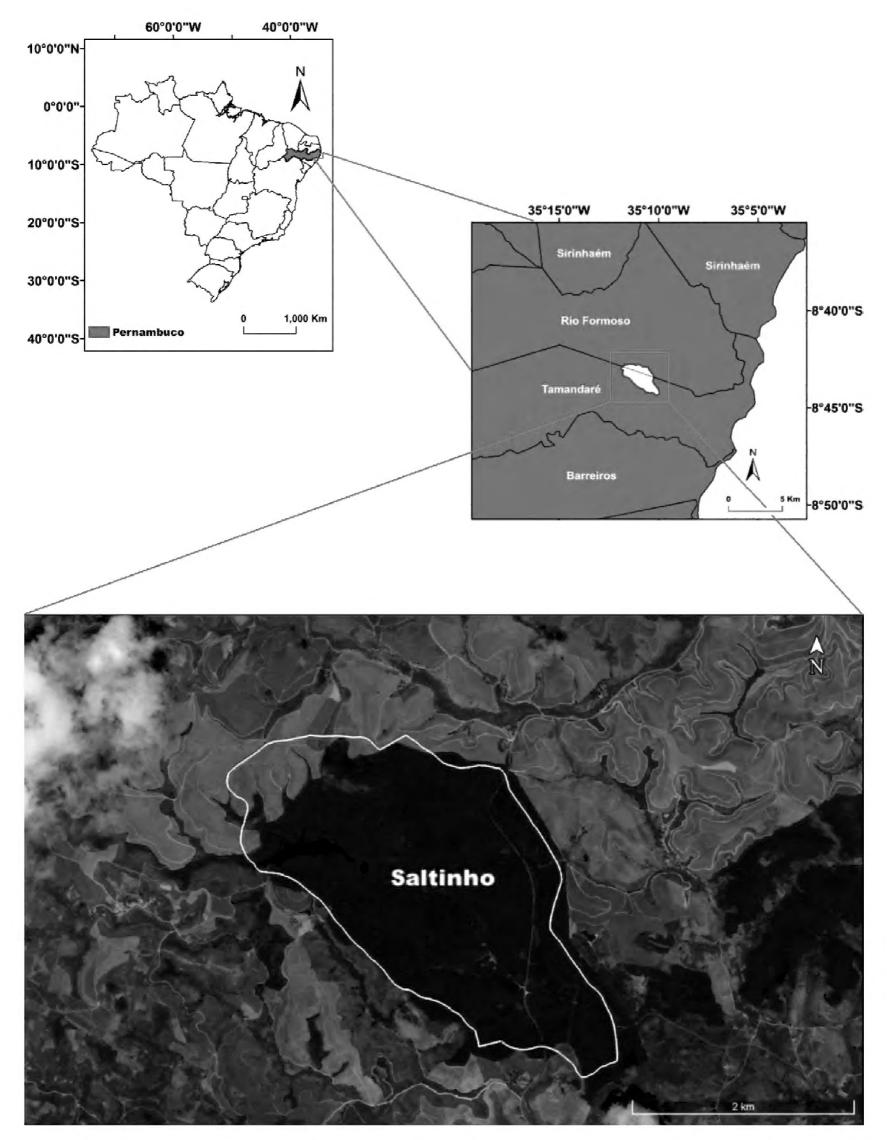


Figure 1. Location of the study area, Reserva Biológica de Saltinho, 08°43′49.32″ S, 035°10′34.95″ W (Google Earth™ Pro satellite imagery, 2016).

in Pernambuco state, Northeastern Brazil (ICMBio 2015). The climate in northeastern Brazil is constantly hot (annual temperature averaging 25°C) and humid with a dry season in the summer and a rainy season (1,500–2,000 mm) along autumn-winter (Barbosa et al. 2002; Ferraz 2002). Composed mainly of secondary forest in advanced natural regeneration, Saltinho was created in 1983, and is currently one of the largest Atlantic Forest fragments of Pernambuco (Brasil 1983; ICMBio 2015).

Data collection

Echolocation calls were recorded along four nights, between 20 November 2014 and 5 December 2014, in 15 min-transects before sunset (60 min in total), along forest edges. Bats were recorded flying alone and flying with conspecifics. Recordings were obtained with the real-time Dodotronic Ultramic™ 200K microphone (www.dodotronic.com) with 200 kHz sampling rate and frequency range up to 100 kHz, linked to an Android™ smartphone (HTC One X). Dodotronic Ultramic was configured to a medium gain amplification level. Calls were recorded using the app USB Audio Recorder Pro™ (www.extreamsd.com) and saved in .wav format.

Echolocation analysis

Echolocation analysis was performed using CallViewer18, a MATLAB based software (Skowronski and Fenton 2008). Spectrogram parameters were set to Fast Fourier Transformation size 1024, windows length equals 1 ms, and a background threshold of 10 dB, using Hanning windows. Using Auto Detection function of CallViewer18, we extracted five variables for each call

detected in a file: call duration (Dur, in ms), minimum frequency (Fmin, in kHz), frequency with maximum energy (FME, in kHz), maximum frequency (Fmax, in kHz), and inter-pulse interval (IPI, in ms) of the calls. These measurements are similar to those taken by other authors as Jung et al. (2007) and Barataud et al. (2013). Auto Detection function parameters were set as minimum link length of six frames, window length 0.3 ms, 10,000 frames per second, chunk size one second, minimum energy equals 15 dB, an echo filter threshold of six dB, upper cutoff frequency of 100 kHz, lower cutoff frequency of 10 kHz, Hanning window type, and delta size equals one frame. Echolocation sequences were played using a time expansion factor of 10. Only sequences containing a minimum of five good quality calls were considered for analysis (Ratcliffe et al. 2011). Feeding buzzes (and calls after and before) or socialcalls were not considered for analysis. Call structure and frequency alternation patterns are valuable for some emballonurid acoustic identifications, such as Saccopteryx species (Ratcliffe et al. 2011). Sonotype identification was made considering the available literature (e.g., Barclay 1983; Jung et al. 2007; Jung and Kalko 2011; Ratcliffe et al. 2011; Barataud et al. 2013). For identification proposes, we also used reference calls from the public call library (Macaulay Library, Cornell Lab 2016), as well some recordings from private call libraries (Michel Barataud, French Guiana; Maria João Pereira, Brazilian Amazon). We used for the discriminant function analysis (DFA) recordings of Peropteryx species from the Caatinga from our personal library. For each species, mean ± standard deviation and range were shown for all analyzed parameters.

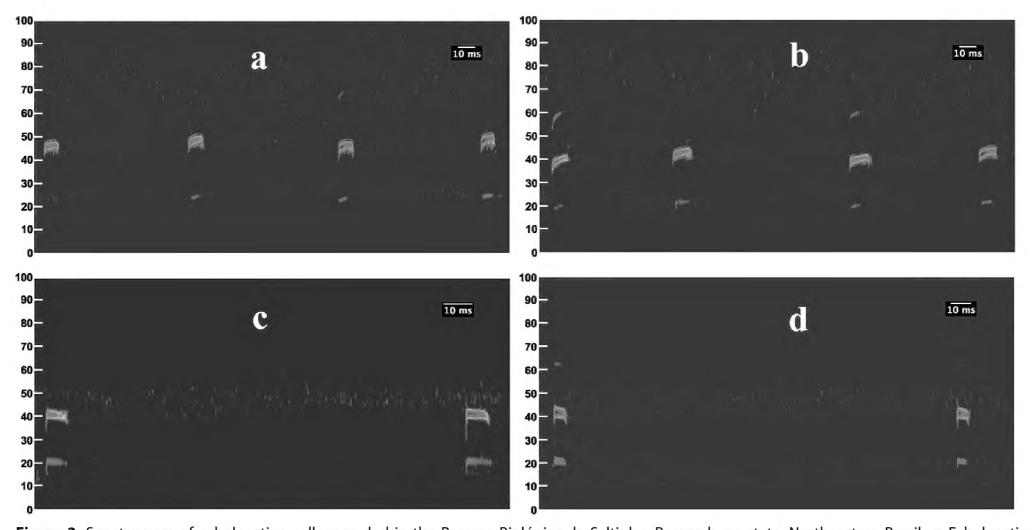


Figure 2. Spectrogram of echolocation calls recorded in the Reserva Biológica de Saltinho, Pernambuco state, Northeastern Brazil. **a:** Echolocation calls of *Saccopteryx bilineata* (sonotype S5). **b:** Echolocation calls of *Saccopteryx sp.* (sonotype S4). **c:** Echolocation calls of *Peropteryx* sp. (sonotype S7). **d:** Echolocation calls of *Centronycteris maximiliani* (sonotype S14). Call's duration and inter-pulse interval were preserved.

Using Graphpad Prism™ 6.0h (for Mac, GraphPad Software, La Jolla, California, USA, www.graphpad. com), we conducted a Kruskal-Wallis with Dunn test to access the variation of the FME between the *Saccopteryx* sonotypes on low and high calls.

Using Past 3.10 (Hammer et al. 2001), DFAs were performed in order to compare mean call parameters of each identified sonotypes with other similar sonotypes from the call libraries cited.

RESULTS

Based on echolocation calls, we found a total of four different emballonurid sonotypes. These were composed by narrowband calls with an evident qCF portion and FME permanently in the second harmonic, all characteristics

typically found in calls from Emballonuridae (Figure 2).

One of these sonotypes (Sonotype S5) was positively matched to *Saccopteryx bilineata* (Temminck, 1838). A second sonotype (Sonotype S4) was identified as also belonging to *Saccopteryx* but could not be identified to species. The third sonotype (Sonotype S7) was identified as *Peropteryx* sp., and the fourth sonotype (Sonotype S14) was identified as *Centronycteris maximiliani* (Fischer, 1829).

Saccopteryx bilineata (Temminck, 1838): Figure 2a Urocryptus bilineatus Temminck, 1838: 33 — E[mballonura] insignis Wagner, 1855: 695; Saccopteryx bilineata, Peters, 1867: 471; Saccopteryx perspicillijer Miller, 1899: 176.

Material examined: Table 1; Figure 2a; Figure 3.

Table 1. Echolocation call characteristics for Emballonuridae sonotypes recorded in Reserva Biológica de Saltinho, Pernambuco state, Northeastern Brazil. Mean ± Standard deviation and the minimum-maximum ranges of the parameters (in parenthesis). FME = frequency with maximum energy, Fmax = maximum frequency, Fmin = minimum frequency, Duration = call duration, IPI = inter-pulse interval, NC(NS) = number of analyzed calls (number of analyzed sequences).

Sonotype	Taxon	FME		Fmin		IPI	
		(kHz)	Fmax (kHz)	(kHz)	Duration (ms)	(ms)	NC(NS)
S5	S. bilineata (low calls)	45.0 ± 0.7 (43.8–46.1)	46.3 ± 0.9 (45.3–47.7)	41.3 ± 1.6 (37.5–43.8)	8.3 ± 1.9 (5.7–13.1)	75.3 ± 11.1 (53.9–91.4) high–low	15(4)
	S. bilineata (high calls)	47.9 ± 0.6 (46.9–49.2)	49.3 ± 0.7 (48.4–50.0)	45.0 ± 0.7 (43.8–46.1)	8.6 ± 1.4 (7.1–12.4)	53.5 ± 12.6 (38.5–74.9) low–high	16(4)
S4	Saccopteryx sp. (low calls)	39.0 ± 0.9 (37.5–40.6)	40.8 ± 0.7 (39.8–42.2)	36.2 ± 2.0 (32.8–38.3)	9.3 ± 1.5 (7.6–12.0)	95.0 ± 11.0 (77.6–109.7) high–low	11(3)
	Saccopteryx sp. (high calls)	42.1 ± 0.4 (41.4–43.0)	43.8 ± 1.1 (42.2–44.5)	40.3 ± 0.6 (39.8–41.4)	9.9 ± 1.6 (7.5–12.9)	63.5 ± 10.1 (51.6–88.9) low–high	12(3)
S7	Peropteryx sp.	41.0± 0.3 (40.5-41.5)	41.6± 0.9 (40.5–43.5)	38.2 ± 0.8 (37.5–39.6)	9.3 ± 1.1 (7.9–10.6)	140.9 ± 29.5 (112.4–214.3)	10(1)
S14	Centronycteris maximiliani	41.5± 0.2 (41.4–42.2)	41.6± 0.3 (41.4–42.2)	38.1 ± 1.1 (36.5–39.6)	5.2 ± 0.4 (4.7-5.9)	154.8 ± 56.9 (108.8-250.4)	10(1)

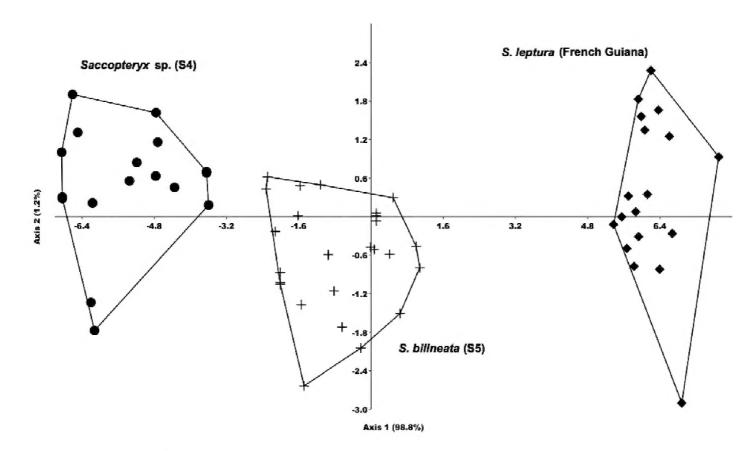


Figure 3. Discriminant function analysis (DFA) using frequencies of maximum energy, minimum frequencies, maximum frequencies and duration values extracted from echolocation calls of *Saccopteryx bilineata* (S5, +) and an unknown species of *Saccopteryx* (S4, ●), both recorded in the Reserva Biológica de Saltinho, Pernambuco State, Northeastern Brazil. For comparison proposes, we included *S. leptura* (♦) sonotype from a bat call library of French Guiana.

The sonotype S5 identified as belonging to this species was characterized by frequency alternation pattern and narrowband echolocation calls, and the qCF portion of the calls had an ascendant modulation, all unambiguous characteristics of *Saccopteryx* species (Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013). The sequences identified as belonging to *S. bilineata* (Figure 2a; Table 1) have alternated frequencies between ~45 kHz and ~48 kHz. The FME was present in the second harmonic of the calls as is typical for all emballonurids (Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013).

Saccopteryx sp.: Figure 2b

Saccopteryx Illiger, 1811: 121 — Vespertilio Schreber, 1774; Urocryptus Temminck, 1838:31

Material examined: Table 1; Figure 2b; Figure 3.

Like S5, sonotype S4 was identified as belonging to *Saccopteryx* and was characterized by frequency alternation pattern (duplets) and narrowband echolocation calls. The qCF portion of the calls had an ascendant modulation and acoustic parameters are very valuable for Saccopteryx species identification (Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013). The FME was present in the second harmonic of the calls as for all emballonurids (Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013). However, these sonotype sequences alternated frequencies between ~39 kHz and ~42 kHz (Figure 2b; Table 1). In fact, *Saccopteryx* is not the only genus of emballonurids that emit alternated pulses. Cormura brevirostris (Wagner, 1843) also uses alternating calls. However, the alternation pattern of Cormura clearly consists of calls emitted in triplets, with frequencies between 26, 29 and 32 kHz (Jung et al. 2007; Barataud et al. 2013). We also excluded the possibility

of our recorded calls to belong to a species of the genus *Diclidurus* Wied-Neuwied, 1820. The lowest frequencies emitted by *Diclidurus* species are around 20 kHz, and the highest around 30 kHz (Jung et al. 2007; Barataud et al. 2013). The frequencies we recorded are between 26% and 36% higher than the highest frequency recorded for *Diclidurus*, which is far from the expected interspecific variation already recorded for any emballonurid. Therefore, we are confident that this sonotype belongs to an unknown cryptic *Saccopteryx* species.

Peropteryx sp.: Figure 2c

Peropteryx Peters, 1867: 469–481 — *Peronymus* Peters, 1868: 145.

Material examined: Table 1; Figure 2c; Figure 4.

The sonotype S7 identified as *Peropteryx* sp. was characterized as monotonous (without frequency alternation) and narrowband calls with a final FM component and slight descending modulation of the qCF portion (Figure 2c). The FME averaged 41 kHz and was present in the second harmonic of the calls as for all emballonurids. The calls also had an average duration of 9 ms (Table 1) (Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013). Data available in the literature (Jung et al. 2007) indicates that calls of P. macrotis (Wagner, 1843) in Panamá and Costa Rica average about 39 kHz (39-41 kHz). According to Barataud et al. (2013), calls for the same species in French Guiana average 38 kHz (37-40 kHz) and those for P. trinitatis Miller, 1899 average 44 kHz (42–44 kHz). Our *Peropteryx* calls averaged 41 kHz (40–41 kHz), which is closer to the calls emitted by *P. macrotis*. Regional variation of calls could explain the differences we observed, but our recordings from *P. macrotis* and *P.* trinitatis in the Caatinga of Pernambuco do not match

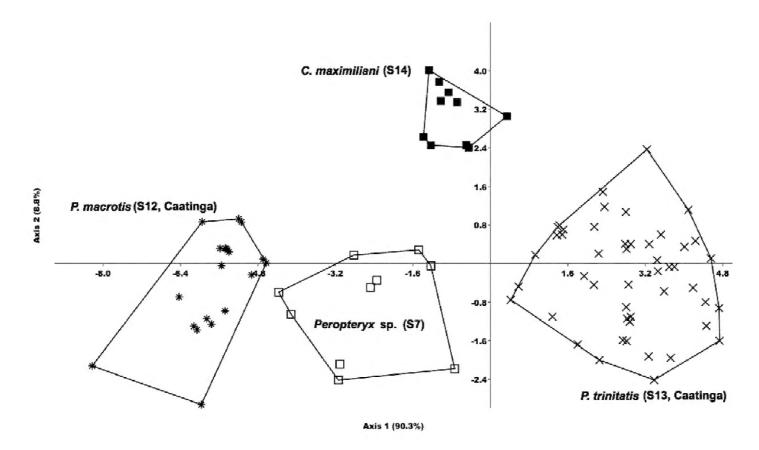


Figure 4. Discriminant function analysis (DFA) using frequencies of maximum energy, minimum frequencies, maximum frequencies and duration values extracted from echolocation calls of *Peropteryx* sp. (S7, ×) *Centronycteris maximiliani* (S14, ■), both recorded in the Reserva Biológica de Saltinho, Pernambuco State, Northeastern Brazil. For comparison proposes, we included *Peropteryx trinitatis* (S13, ×) and *P. macrotis* (S12, −) sonotypes from our bat call library of Caatinga biome.

with this *Peropteryx* sonotype (Figure 4). Considering the relation between frequency and body mass, it is plausible that this sonotype belongs to *P. pallidoptera* Lim, Engstrom, Reid, Simmons, Voss & Fleck, 2010 or *P. leucoptera* Peters, 1867, species for which their body masses are between *P. macrotis* and *P. trinitatis* (Bogdanowicz et al. 1999; Jung et al. 2007; Lim et al. 2010). However, the possibility that this sonotype belongs to *P. macrotis* cannot be disregarded; we were not confident in identifying this sonotype to species.

Centronycteris maximiliani (Fisher, 1829): Figure 2d Vespertilio maximiliani Fischer, 1829: 112–113 — Vespertilio calcaratus Schinz, 1821: 180; Vespertilio maximiliani Fischer, 1829: 112–113; Emballonura calcarata Temminck, 1841: 299; Proboscidea calcarata Gray, 1838: 499; Centronycteris calcarata Gervais, 1856: 69; Saccopteryx calcarata Dobson, 1878: 376; Saccopteryx wiedi Palmer, 1898: 110; Centronycteris wiedi Trouessart, 1904: 98; Centronycteris maximiliani Miller, 1907: 91.

Material examined: Table 1; Figure 2d; Figure 4.

The sonotype S14, identified as belonging to *C. maximiliani*, was characterized by monotonous and narrowband calls with marked initial and final FM components and a straight modulation of the qCF portion (Figure 2d). The FME averaged at 41 kHz and was present in the second harmonic of the calls as for all emballonurids, and the calls also had an average duration of 5 ms (Table 1) (Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013).

Statistically significant differences were found between FME for both Saccopteryx sonotypes (H = 50.12, p < 0.0001). Dunn's comparisons confirmed significant differences between the FME of the lower (Diff = 25.0, p < 0.05) and higher calls (Diff = 29.0, p < 0.0001) for both sonotypes. As expected, no differences were found between the FME of the calls of the same taxa (S.bilineata: Diff = 13.5, p = 0.055; Saccopteryx sp.: Diff = 11.5, p = 0.468). In comparison with the S.leptura (Schreber, 1774) sonotype, the DFA confirmed two distinct groups based on the echolocation parameters of S.bilineata and the second Saccopteryx sonotype (Figure 3). We selected 31 calls from four sequences of S.bilineata and 23 calls from three sequences of the unknown recorded Saccopteryx sp. for a detailed analysis (Table 1).

In comparison with *Peropteryx trinitatis* and *P. macrotis* sonotypes, the DFA performed confirmed distinct groups based on the echolocation parameters of *C. maximiliani* sonotype (Sonotype S14) and the *Peropteryx* sonotype (Sonotype S7) (Figure 4). We selected 10 calls from one sequence of *Peropteryx* sp. and 10 calls from one sequence of *C. maximiliani* for a detailed analysis (Table 1).

DISCUSSION

Currently, in Pernambuco state, there are records of captured individuals of *Saccopteryx bilineata* and *S. leptura* (Guerra 2007), but so far none had their calls

recorded. Relying on the patterns and characteristics of echolocation calls, we believe that we recorded the presence of a third *Saccopteryx* species in the state, a possible undescribed cryptic species for Northeastern Brazil. This sonotype presented the same echolocation patterns as *S. bilineata*, but with lower frequencies (e.g., Jung et al. 2007; Ratcliffe et al. 2011; Barataud et al. 2013). Moreover, our recordings also indicate the presence of *S. bilineata* in sympatry with the unknown sonotype, demonstrating this sonotype should not be perceived as a regional variation of *S. bilineata* calls.

Considering the negative correlation between body size and call frequency observed in bats (e.g., Bogdanowicz et al. 1999; Jung et al. 2007), it is very likely that this unknown sonotype was emitted by a species larger than *S. bilineata*. Therefore, this evidence dismisses that this new sonotype belongs to *S. leptura*, *S. gymnura* Thomas, 1901 or *S. canescens* Thomas, 1901 because these species are smaller bodied than *S. bilineata* (Yancey et al. 1998; Gardner 2008).

The possibility of new cryptic species in the genus Saccopteryx has been previously recognized. Studies using DNA barcoding for specimens from Central America and northern South America revealed that S. bilineata has three different intraspecific mtDNA lineages that strongly support the existence of cryptic species (Clare 2011; Clare at al. 2011). Considering no specimens from the Atlantic Forest were analyzed in those studies, the possibility of additional cryptic species is possible. Interestingly, the acoustic parameters exhibited by the *S. bilineata* sonotype recorded in Pernambuco were closer to Central America sonotypes than those from nearby areas, such as French Guiana or northern Brazil (Barclay 1983; Jung et al. 2007; Jung and Kalko 2011; Ratcliffe et al. 2011; Barataud et al. 2013). The reason behind such an unusual find remains unclear, but with the evidence presented here of a possible cryptic species, the morphology and genetics of specimens of S. bilineata in collections from Northeastern Brazil must be made. Several of recently described bat species were stored and misidentified in collections for several years (e.g., Gregorin and Ditchfield 2005; Dias et al. 2013; Moratelli and Wilson 2014), and it would not be surprising to find that this unknown *Saccopteryx* species was similarly overlooked.

The only previous record of *C. maximiliani* in Pernambuco was a female collected in Saltinho in 1978 (Guerra 2007). Therefore, the recorded sonotype of *C. maximiliani* is important, not only because it is the first acoustic record of this species for Pernambuco, but also because it confirms that this poorly known species remains present in the reserve after 37 years. Although widespread across South America, *C. maximiliani* is rarely captured with net sampling; currently there are only 14 records of captures in Brazil, but only three of

those records were not in the Amazonian biome (Rocha et al. 2015).

The identification of the recorded *Peropteryx* sonotype to species is challenging. The recorded calls are between the frequencies described for P. macrotis and P. trinitatis (Jung et al. 2007; Barataud et al. 2013), averaging 41 kHz, which is closer to the calls emitted by P. macrotis, However, our DFA clearly separated our calls to those belonging to other species, with no overlap with *P. macrotis* nor *P. trinitatis*. Although the possibility of P. macrotis cannot be disregarded, by exclusion and considering the negative correlation between body size and call frequency (e.g., Bogdanowicz et al. 1999; Jung et al. 2007) this sonotype may belong to P. leucoptera or P. pallidoptera. Actually, the only record of P. pallidoptera in Brazil was in Pará state (Nogueira et al. 2014). Guerra (2007) reported P. leucoptera for Pernambuco, in the Dois Irmãos Estadual Park and in São Lourenço da Mata and Rio Formoso municipalities, all in Atlantic Forest.

Bioacoustics devices are now affordable (Rydell et al. 2002; Adams et al. 2012) and complement netting efforts (O'Farrell and Gannon 1999; Sampaio et al. 2003; Kunz and Parsons 2009). Most emballonurids, vespertilionids, and molossids are easily detected by their echolocation calls but are difficult to sample using netting (Rydell et al. 2002). Our results emphasize the importance of acoustic records in bat inventories across Brazil, especially to assess hard-to-capture species.

Moreover, we identified a possible cryptic species using bioacoustics. The identification of cryptic species is a challenge to science, especially at a time when the biodiversity of the planet is threatened (Pfenninger and Schwenk 2007). Inaccurate identifications may hamper real estimates of species richness and diversity in any given habitat and consequently affect our understanding on how much of this biodiversity is threatened. A study detailing the taxonomic composition of 408 new mammal species described since 1993, found that about 60% of these species were cryptic, and bats accounted for 23% of these (Ceballos and Ehrlich 2009). For bats, the number of new species is greater than expected, and the potential for new species is great and the list of known species far from complete (Reeder et al. 2007; Ceballos and Ehrlich 2009; Clare et al. 2011). Most of these new mammalian species have been described in South America and Asia and about 12% of these occurrences are where the landscape is dominated (> 50%) by agriculture, while 20% were in regions with relatively high human population densities, that imply these species are highly vulnerable to anthropogenic threats (Ceballos and Ehrlich 2009). Different species may have differing conservation demands and correct identification of cryptic species may have direct consequences for conservation planning: some endangered taxa may be in fact a complex of multiple

cryptic species, with some of them even more rare or endangered than previously supposed (e.g., Bowen et al. 1993; Ravaoarimanana et al. 2004). This seems to hold true for the unknown *Saccopteryx* we recorded. Most of the Atlantic Forest in Pernambuco is gone, with around 12% of the original forest cover remaining (SOS-MA/INPE 2013). The Reserva Biológica Saltinho, the site of the recordings is a 562 ha fragment of mixed vegetation completely surrounded by a matrix of sugarcane plantations. If this *Saccopteryx* proves to be a new species, its conservation scenario is already alarming.

ACKNOWLEDGEMENTS

We thank Mariana Delgado Jaramillo and Ana Cláudia Jardelino for their valuable help during fieldwork in Saltinho. We thank Maria João Pereira, Michel Barataud and the Macaulay Library at the Cornell Lab of Ornithology for the reference recordings used in the DFAs. We also thank the Department of Zoology at the Universidade Federal de Pernambuco, for supporting our work on Brazilian bats. E. Bernard is supported by a CNPq productivity grant and E. Barbier is supported by a CAPES PhD grant.

LITERATURE CITED

Adams, A.M., M.K. Jantzen, R.M. Hamilton and M.B. Fenton. 2012. Do you hear what I hear? Implications of detector selection for acoustic monitoring of bats. Methods in Ecology and Evolution 3(4): 992–998. doi: 10.1111/j.2041-210X.2012.00244.x

Barataud, M., S. Giosa, F. Leblanc, V. Rufray, T. Disca, L. Tillon, M. Delaval, A. Haquart and M. Dewynter. 2013. Identification et écologie acoustique des chiroptères de Guyane Française. Le Rhinolophe 19: 103–145. http://ecologieacoustique.fr/?page_id=11

Barbosa, D.C.A., M.C.A. Barbosa and P.G.G. Silva. 2002. Tipos de frutos e síndromes de dispersão de espécies lenhosas da Caatinga de Pernambuco; pp. 609–621, in: M. Tabarelli and J.M.C. Silva (eds.). Diagnóstico da biodiversidade de Pernambuco, Volume 2. Recife: SECTMA and Editora Massangana.

Barclay, R.M.R. 1983. Echolocation calls of emballonurid bats from Panama. Journal of Comparative Physiology 151(4): 515–520. doi: 10.1007/BF00605468

Bogdanowicz, W., M.B. Fenton and K. Daleszczyk. 1999. The relationships between echolocation calls, morphology and diet in insectivorous bats. Journal of Zoology 247(3): 381–393. doi: 10.1111/j.1469-7998.1999.tb01001.x

Bowen, B.W., W.S. Nelson and J.C. Avise. 1993. A molecular phylogeny for marine turtles: trait mapping, rate assessment, and conservation relevance. Proceedings of the National Academy of Sciences of the United States of America 90(12): 5574–5577. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC46763/

Brasil. 1983. Reserva Biológica de Saltinho. Decreto de criação N° 88.744. Accessed at http://www.icmbio.gov.br/portal/images/stories/imgs-unidades-coservacao/rebio_saltinho.pdf, 9 November 2015.

Britton, A.R. and G. Jones 1999. Echolocation behaviour and prey-capture success in foraging bats: laboratory and field experiments on *Myotis daubentonii*. The Journal of Experimental Biology 202(13): 1793–801. http://www.ncbi.nlm.nih.gov/pubmed/10359682

Ceballos, G. and P.R. Ehrlich. 2009. Discoveries of new mammal species and their implications for conservation and ecosystem

- services. Proceedings of the National Academy of Sciences 106(10): 3841–3846. doi: 10.1073/pnas.0812419106
- Clare, E.L. 2011. Cryptic Species? Patterns of maternal and paternal gene flow in eight Neotropical bats. PLoS ONE 6(7): e21460. doi: 10.1371/journal.pone.0021460
- Clare, E.L., B. K. Lim, M.B. Fenton and P.D.N. Hebert. 2011. Neotropical bats: estimating species diversity with DNA barcodes. PLoS ONE 6(7): e22648. doi: 10.1371/journal.pone.0022648
- Cornell Lab. 2016. Macaulay Library at the Cornell Lab of Ornithology. Cornell Lab of Ornithology. Accessed at http://macaulaylibrary.org/, 18 January 2016.
- Dias, D., C.E.L. Esbérard and R. Moratelli. 2013. A new species of *Lonchophylla* (Chiroptera, Phyllostomidae) from the Atlantic Forest of southeastern Brazil, with comments on *L. bokermanni*. Zootaxa 3722(3): 347–360. doi: 10.11646/zootaxa.3722.3.4
- Estrada, A., E. Fuentes, C. Jiménez and A. Rivera. 2004. General bat activity measured with an ultrasound detector in a fragmented tropical landscape in Los Tuxtlas, Mexico. Animal Biodiversity and Conservation 27(2):5–13. http://abc.museucienciesjournals.cat/volum-27-2-2004-abc/?lang=en
- Ferraz, E.M.N. 2002. Panorama da Floresta Atlântica no estado de Pernambuco; pp. 23–26, in: E.L. Araújo, A.N. Moura, E.V.S.B. Sampaio, L.M.S. Gestinari and J.M.T. Carneiro (eds.). Biodiversidade, conservação e uso sustentável da flora do Brasil. Recife: Imprensa Universitária/UFRPE.
- Fischer, J.B. 1829. Synopsis mammalium. Stuttgart: J.G. Cottae. 752 pp.
- Ford, W.M., J.M. Menzel, M.A. Menzel, J.W. Edwards and J.C. Kilgo. 2006. Presence and absence of bats across habitat scales in the upper coastal plain of South Carolina. Journal of Wildlife Management 70(5): 1200–1209. doi: 10.2193/0022-541X(2006)70[1200:PAAOBA]2.0.CO;2
- Gardner, A.L. 2008. Mammals of South America, Volume 1: marsupials, xenarthrans, shrews, and bats. Chicago: University of Chicago Press. 669 pp.
- Goodman, S.M., S.G. Cardiff, J. Ranivo, A.L. Russell and A.D. Yoder. 2006. A new species of *Emballonura* (Chiroptera: Emballonuridae) from the dry regions of Madagascar. American Museum Novitates 3538: 1–24. doi: 10.1206/0003-0082(2006)3538[1:ANSOEC]2.0 .CO;2
- Goodman, S.M., S.G. Cardiff and F.H. Ratrimomanarivo. 2008. First record of *Coleura* (Chiroptera: Emballonuridae) on Madagascar and identification and diagnosis of members of the genus. Systematics and Biodiversity 6(2): 283–292. doi: 10.1017/S1477200008002715
- Gregorin, R. and A.D. Ditchfield. 2005. New genus and species of nectar-feeding bat in the tribe Lonchophyllini (Phyllostomidae: Glossophaginae) from Northeastern Brazil. Journal of Mammalogy 86(2): 403–414. doi: 10.1644/BRB-229.1
- Greif, S. and B.M. Siemers. 2010. Innate recognition of water bodies in echolocating bats. Nature Communications 1: 107. doi: 10.1038/ncomms1110
- Guerra, D.Q. 2007. Chiroptera de Pernambuco: distribuição e aspectos biológicos [M.Sc. dissertation]. Pernambuco: Universidade Federal de Pernambuco. 103 pp.
- Hammer, O., D. Harper and P. Ryan. 2001. PAST. Palaeontological statistics. Accessed at http://folk.uio.no/ohammer/past/, 18 January 2016.
- Horta, P., H. Raposeira, H. Santos, P. Alves, J. Palmeirim, R. Godinho, G. Jones and H. Rebelo. 2015. Bats' echolocation call characteristics of cryptic Iberian *Eptesicus* species. European Journal of Wildlife Research 61(6): 813–818. doi: 10.1007/s10344-015-0957-x
- ICMBio. 2015. Instituto Chico Mendes de Conservação da Biodiversidade. Reserva Biológica de Saltinho. Accessed at http://www.icmbio.gov.br/portalbiodiversidade/unidades-de-conserv

- acao/biomas-brasileiros/mata-atlantica/unidades-de-conservacao-mata-atlantica/2156-rebio-de-saltinho.html, 10 November 2015.
- Illiger, C. 1811. Prodromus systematis mammalium et avium. Berlin: Sumptibus C. Salfied. 302 pp. doi: 10.5962/bhl.title.42403
- Jacobs, D.S. and R.M.R. Barclay. 2009. Niche differentiation in two sympatric sibling bat species, *Scotophilus dinganii* and *Scotophilus mhlanganii*. Journal of Mammalogy 90(4): 879–887. doi: 10.1644/08-MAMM-A-235.1
- Jacobs, D.S., G.N. Eick, M.C. Schoeman and C.A. Matthee. 2006. Cryptic species in an insectivorous bat, *Scotophilus dinganii*. Journal of Mammalogy 87(1):161–170. doi:10.1644/04-MAMM-A-132R2.1
- Jones, G. and E. Barratt. 1999. Vespertilio pipistrellus Schreber, 1774 and V. pygmaeus Leach, 1825 (currently Pipistrellus pipistrellus and P. pygmaeus; Mammalia, Chiroptera): proposed designation of neotypes. Bulletin of Zoological Nomenclature 56(3): 182–186. doi: 10.5962/bhl.part.23065
- Jones, G. and E. Teeling. 2006. The evolution of echolocation in bats. Trends in Ecology & Evolution 21(3): 149–156. doi: 10.1016/j. tree.2006.01.001
- Jones, G. and S.M.V. Parijs. 1993. Bimodal echolocation in pipistrelle bats: are cryptic species present? Proceedings of the Royal Society of London B 251: 119–125. doi: 10.1098/rspb.1993.0017
- Jung, K. and E.K.V. Kalko. 2011. Adaptability and vulnerability of high flying Neotropical aerial insectivorous bats to urbanization. Diversity and Distributions 17(2): 262–274. doi: 10.1111/j.1472-4642.2010.00738.x
- Jung, K., E.K. Kalko and O. von Helversen. 2007. Echolocation calls in Central American emballonurid bats: signal design and call frequency alternation. Journal of Zoology 272(2): 125–137. doi: 10.1111/j.1469-7998.2006.00250.x
- Kalko, E.K. 1995. Echolocation signal design, foraging habitats and guild structure in six Neotropical sheath-tailed bats (Emballonuridae). Symposia of the Zoological Society of London 67: 259–273. http://collections.si.edu/search/results.htm?q=record_ID:SILSRO_97467
- Kunz, T.H. and S. Parsons. 2009. Ecological and behavioral methods for the study of bats, Second Edition. Washington, DC: Smithsonian Institution Press. 920 pp.
- Lim, B.K., M.D. Engstrom, F.A. Reid, N.B. Simmons, R.S. Voss and D.W. Fleck. 2010. A new species of *Peropteryx* (Chiroptera: Emballonuridae) from Western Amazonia with comments on phylogenetic relationships within the genus. American Museum Novitates 3686: 1–20. doi: 10.1206/691.1
- Moratelli, R. and D.E. Wilson. 2014. A new species of *Myotis* (Chiroptera, Vespertilionidae) from Bolivia. Journal of Mammalogy 95(4): e17–e25. doi: 10.1644/14-MAMM-149
- Nogueira, M.R., I.P. Lima, R. Moratelli, V. Tavares, R. Gregorin and A.L. Peracchi. 2014. Checklist of Brazilian bats, with comments on original records. Check List 10(4): 808–821. doi: 10.15560/10.4.808
- O'Farrell, M.J. and W.L. Gannon. 1999. A comparison of acoustic versus capture techniques for the inventory of bats. Journal of Mammalogy 80(1): 24–30. doi: 10.2307/1383204
- Peters, W. 1867. Über die zu den gattungen *Mimon* und *Saccopteryx* gehörigen fledertiere. Monatsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin 1867: 469–481. http://biodiversitylibrary.org/page/36510740
- Peters, W. 1868. Über eine neue Untergattung der Flederthiere, so wie über neue Gattungen und Arten von Fischen. Monatsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin 1869: 145–148. http://www.biodiversitylibrary.org/page/35989867
- Pfenninger, M. and K. Schwenk. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evolutionary Biology 7: 121. doi: 10.1186/1471-2148-7-121

- Ramasindrazana, B., S.M. Goodman, M.C. Schoeman and B. Appleton. 2011. Identification of cryptic species of *Miniopterus* bats (Chiroptera: Miniopteridae) from Madagascar and the Comoros using bioacoustics overlaid on molecular genetic and morphological characters. Biological Journal of the Linnean Society 104(2): 284–302. doi: 10.1111/j.1095-8312.2011.01740.x
- Ratcliffe, J.M., L. Jakobsen, E.V. Kalko and A. Surlykke. 2011. Frequency alternation and an offbeat rhythm indicate foraging behavior in the echolocating bat, *Saccopteryx bilineata*. Journal of Comparative Physiology A 197(5): 413–423. doi: 10.1007/s00359-011-0630-0
- Ravaoarimanana, I.B., R. Tiedemann, D. Montagnon and Y. Rumpler. 2004. Molecular and cytogenetic evidence for cryptic speciation within a rare endemic Malagasy lemur, the Northern Sportive Lemur (*Lepilemur septentrionalis*). Molecular Phylogenetics and Evolution 31(2): 440–448. doi: 10.1016/j.ympev.2003.08.020
- Reeder, D.M., K.M. Helgen and D.E. Wilson. 2007. Global trends and biases in new mammal species discoveries. Lubbock: Museum of Texas Tech University. 35 pp.
- Rocha, P.A., M.V. Brandão, A.C.D. Oliveira Júnior and C.C. Aires. 2015. Range extension of *Centronycteris maximiliani* (Mammalia: Chiroptera) for southern Amazonia. Acta Amazonica 45(4): 425–430. doi: 10.1590/1809-4392201501131
- Russo, D. and G. Jones. 2002. Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. Journal of Zoology 258(1): 91–103. doi: 10.1017/S0952836902001231
- Rydell, J., H. Arita, M. Santos and J. Granados. 2002. Acoustic identification of insectivorous bats (order Chiroptera) of Yucatan, Mexico. Journal of Zoology 257(1): 27–36. doi: 10.1017/S0952836902000626
- Sampaio, E.M., E.K. Kalko, E. Bernard, B. Rodríguez-Herrera and C.O. Handley. 2003. A biodiversity assessment of bats (Chiroptera) in a tropical lowland rainforest of Central Amazonia, including methodological and conservation considerations. Studies on Neotropical Fauna and Environment 38(1): 17–31. doi: 10.1076/snfe.38.1.17.14035
- Sattler, T., F. Bontadina, A. H. Hirzel and R. Arlettaz. 2007. Ecological niche modelling of two cryptic bat species calls for a reassessment of their conservation status. Journal of Applied Ecology 44(6): 1188–1199. doi: 10.1111/j.1365-2664.2007.01328.x

- Simmons, N.B., D. Wilson and D. Reeder. 2005. Order Chiroptera; pp. 312–529, in D. Wilson and D. Reeder (eds.). Mammal species of the world: a taxonomic and geographic reference. Baltimore: Johns Hopkins University Press.
- Skowronski, M.D. and M.B. Fenton. 2008. Model-based automated detection of echolocation calls using the link detector. The Journal of the Acoustical Society of America 124(1): 328–336. doi: 10.1121/1.2924122
- SOS-MA/INPE. 2013. Fundação SOS Mata Atlântica/Instituto Nacional de Pesquisas Espaciais. Atlas dos remanescentes florestais da Mata Atlântica: período de 2011 a 2012. Accessed at https://www.sosma.org.br/wp-content/uploads/2013/06/atlas_2011-2012_relatorio_tecnico_2013final.pdf, 10 November 2015.
- Sztencel-Jablonka, A., G. Jones and W. Bogdanowicz. 2009. Skull morphology of two cryptic bat species: *Pipistrellus pipistrellus* and *P. pygmaeus* a 3D geometric morphometrics approach with landmark reconstruction. Acta Chiropterologica 11(1): 113–126. doi: 10.3161/150811009X465730
- Temminck, C.J. 1838. Over de geslachten Taphozous, Emballonura, Urocryptus en Diclidurus. Tijdschrift Voor Natuurlijke Geschiedenis en Physiologie 5: 1–34. http://biodiversitylibrary.org/page/13474775
- Thoisy, B.D., A.C. Pavan, M. Delaval, A. Lavergne, T. Luglia, K. Pineau, M. Ruedi, V. Rufray and F. Catzeflis. 2014. Cryptic diversity in common mustached bats *Pteronotus* cf. *parnellii* (Mormoopidae) in French Guiana and Brazilian Amapa. Acta Chiropterologica 16(1): 1–13. doi: 10.3161/150811014X683228
- von Helversen, O., K.G. Heller, F. Mayer, A. Nemeth, M. Volleth and P. Gombkötö. 2001. Cryptic mammalian species: a new species of whiskered bat (*Myotis alcathoe* n. sp.) in Europe. Naturwissenschaften 88(5): 217–223. doi: 10.1007/s001140100225
- Yancey, F.D., J.R. Goetze and C. Jones. 1998. Saccopteryx bilineata. Mammalian Species 581: 1–5. doi: 10.2307/3504459

Author contributions: F. Hintze and E. Barbier collected the data; F. Hintze, E. Barbier and E. Bernard wrote the manuscript text; and F. Hintze made the bioacoustics analysis.

Received: 5 March 2016 Accepted: 10 June 2016 Academic editor: Sergio Solari